Research

Kindling-induced changes in plasticity of the rat amygdala and hippocampus

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Temporal lobe epilepsy (TLE) is often accompanied by interictal behavioral abnormalities, such as fear and memory impairment. To identify possible underlying substrates, we analyzed long-term synaptic plasticity in two relevant brain regions, the lateral amygdala (LA) and the CA1 region of the hippocampus, in the kindling model of epilepsy. Wistar rats were kindled through daily administration of brief electrical stimulations to the left basolateral nucleus of the amygdala. Field potential recordings were performed in slices obtained from kindled rats 48 h after the last induced seizure, and in slices from sham-implanted and nonimplanted controls. Kindling resulted in a significant impairment of long-term potentiation (LTP) in both the LA and the CA1, the magnitude of which was dependent on the number of prior stage V seizures. Saturation of CA1-LTP, assessed through repeated spaced delivery of high-frequency stimulation, occurred at lower levels in kindled compared to sham-implanted animals, consistent with the hypothesis of reduced capacity of further synaptic strengthening. Furthermore, theta pulse stimulation elicited long-term depression in the amygdala in nonimplanted and sham-implanted controls, whereas the same stimulation protocol stimulation caused LTP in kindled rats. In conclusion, kindling differentially affects the magnitude, saturation, and polarity of LTP in the CA1 and LA, respectively, most likely indicating an activity-dependent mechanism in the context of synaptic metaplasticity.

Convulsive diseases in humans such as temporal lobe epilepsy (TLE) are often accompanied by impairments of learning and memory. In addition, TLE patients typically display emotional disturbances, in particular negative emotions such as fear, anxiety, and depression (Kalynchuk 2000). A wealth of data has established that the amygdala plays a critical role in neuronal activity and synaptic plasticity related to both emotionally modulated signal processing and epilepsy (Gloor 1990; Maren 1999; LeDoux 2000). Synapses in the amygdala display long-term potentiation (LTP) (Chapman and Chattarji 2000; LeDoux 2000; Maren 2001), a long-lasting increase of synaptic strength that is widely believed to be a cellular basis of learning and memory (Bliss and Collingridge 1993). The amygdala possesses the lowest threshold for the induction of kindling (Löscher 1997), an established experimental model of TLE in which daily electrical stimulation results in a gradual progression and intensification of limbic motor seizures (Goddard et al. 1969). Although kindling has been extensively investigated in the context of its clinical relevance to epilepsy and seizure-induced synaptic potentiation, only a few studies that relate to plastic synaptic changes after kindling are available. Therefore, the present study was undertaken to systematically analyze the influence of kindling 48 h after the last seizure on long-term synaptic plasticity, LTP, and long-term depression (LTD) in the lateral nucleus of the amygdala (LA) in rats. The LA was chosen because it represents the major input station for sensory signals to the amygdala and for reciprocal interconnections with the hippocampus (Pitkänen et al. 1997). For comparison, synaptic plasticity was assessed in the CA1 region of the hippocampus in the same animals. All experiments were performed in combined horizontal brain slices ex vivo, in which a majority of synaptic interconnections within the amygdala and between the amygdala, the hippocampus, and the entorhinal cortex are preserved (von Bohlen und Halbach and Albrecht 1998, 2002).

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Materials and Methods

Animals and treatment

Four groups of male Wistar rats (6-wk-old, 180–210 g) were used to assess kindling-related effects and to control for the effects of electrode implantation (Löscher et al. 1993, 1995): age-matched nonimplanted controls ($n = 29$), age-matched sham-implanted controls ($n = 20$), and two kindled groups of rats ($n = 23$). Animals were housed under standard laboratory conditions (22° ± 1°C, 60%–65% relative humidity, 12 h light/12 h dark alternate cycles, food and water ad libitum).

Rats were anesthetized with ketamine/rompune (87 mg/kg and 13 mg/kg, i.p.). Bipolar stainless steel electrodes were implanted into the basolateral nucleus of the left amygdala (AP −2.3, L 5.0, H 8.5) (Paxinos and Watson 1986). Proper electrode location was histologically verified in all animals. After a postsurgical recovery period of 7–10 d, one group (K7) was stimulated daily (5 ×/wk) through the implanted electrode by a train of pulses (duration 0.1 msec) at 60 Hz for 1 sec (300–450 μA). The intensity used for stimulation was set to initially induce only a twitching of the eye ipsilateral to the stimulated amygdala. This threshold current was kept constant and delivered 1 ×/d until seven consecutive stage V seizures were evoked. The second group of animals (K15) was stimulated 2 ×/d until 15 consecutive stage V seizures were evoked. Behavioral changes during kindling were scored according to the scale of Racine (Racine et al. 1972). A mean of 10 ± 1 (K7; $n = 19$) and 16 ± 2 (K15; $n = 4$) stimulations, respectively, was required to evoke the first stage V seizure, i.e., performing the kindling procedure twice daily needed more time than kindling once a day (Tuunanen et al. 1997; Eskazan et al. 2003). On these last seven or 15 stimulations, the mean duration of seizures was 52.0 ± 4.3 sec and 53.0 ± 13.3 sec, respectively. The mean age of the rats, at the

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time of the ex-vivo experiments (nonimplanted = 82 ± 4 d; sham-implanted = 81 ± 4 d; K7 = 85 ± 3 d; K15 = 78 ± 4 d) was similar among the different groups. The weights of the sham-implanted and kindled groups were measured daily after electrode implantation. Regarding the body weights at the day of the in vitro experiment, those of the nonimplanted and sham-implanted rats did not differ significantly (358 ± 10 g vs. 376 ± 10 g, respectively; n.s.), whereas there were significant differences between the sham-implanted and kindled animals (K7, 413 ± 8 g; $P = 0.02$; K15, 461 ± 7 g; $P = 0.002$) and between the K7 and K15 groups ($P = 0.008$) in accord with data from the literature (Adamec et al. 2004). All experiments were carried out in accord with the European Communities Council Directive of 24 November 1986 (89/609/EEC) and approved by the regional Berlin animal ethics committee (G0291/01). All efforts were made to minimize suffering and the numbers of animals used.

Preparation and recording

The rats were anesthetized with ether and decapitated. Their brains were rapidly removed and placed in ice-cold carbogenated artificial cerebrospinal fluid (ACSF) of the following composition: NaCl, 124 mM; KCl, 3 mM; $NaHCO_3$, 26 mM; Na_2HPO_4, 1.25 mM; $MgSO_4$, 1.8 mM; $CaCl_2$, 1.6 mM; glucose, 10 mM. The cerebellum was removed and a cut was made to divide the two cerebral hemispheres. Each hemisphere was fixed onto the persepex carrier of a vibroslicer (Campden Instruments) using a cyanoacrylat adhesive (Pattex). Horizontal slices (400-µm thick) containing the amygdala, the hippocampus, the entorhinal cortex, and parts of the piriform cortex were prepared (von Bohlen und Halbach and Albrecht 1998, 2002). The slices were placed in an interface chamber and allowed to equilibrate for 120 min at 34°C. They were superfused continuously with ACSF (1.5 mL/min). The pH was maintained at 7.4 by carbogenating the solution with 95% O_2 and 5% CO_2. Extracellular recordings from the LA and CA1 region were made simultaneously in different slices.

Glass microelectrodes (GB 120F-10, Science Products) were filled with ACSF (tip resistances 1 MΩ) and placed either in the stratum radiatum of the CA1 region or in the caudoventral part of the LA to record field excitatory postsynaptic potential (fEPSP) amplitude. Bipolar stimulation electrodes were used to stimulate either Schaffer collaterals or afferents running within the LA. Single stimuli (duration 100 µsec) were presented every 10 sec. Signals of the evoked responses were amplified and filtered (bandpass: 0.1 Hz to 3 KHz) by a pre-amplifier (World Precision Instruments), displayed on a storage oscilloscope and fed via a CED laboratory interface (Cambridge Electronic Design) to a computer for storage. The experimenter was not aware of the history of stimulation of each rat, and ex vivo experiments were randomized with respect to the different groups of animals on a day-to-day basis.

Stimulation parameters

An input/output (I/O) response curve was constructed by varying the intensity of single-pulse stimulation, and averaging six responses to each intensity. In the CA1, the intensity was set to evoke a negative response in stratum radiatum not contaminated by a population spike. Because of the different architecture of the LA and CA1, the stimulus intensity that evoked an fEPSP amplitude equal to 50% of the maximal response (LA) or a mean fEPSP amplitude equal to 30% of the maximum response (CA1) was then used for all subsequent stimulations, i.e., high-frequency stimulation (HFS), low-frequency stimulation (LFS), theta pulse stimulation (TPS), and subsequent single-pulse stimulations.

Single stimuli were applied for at least 30 min, and responses were monitored. Once a stable baseline of responses had been obtained for at least 20 min, in the first part of the experiments HFS was delivered as two trains at 100 Hz (1-sec duration; 30 sec apart). Subsequent responses to single stimuli were recorded for at least 60 min, and their amplitude was quantified as percent change with respect to baseline. We used HFS because in the preparation for this study we found that theta burst stimulation did not induce reliable LTP in the LA in older rats (>8-wk-old). To test saturation of LTP, HFS was applied four times. The time interval between multiple spaced HFS was 20 min. In different animals, either LFS (1 Hz, 900 pulses) or TPS (8 Hz, 150 sec) was applied. Reversal of TPS-induced LTD was tested by application of HFS in accord with our previous results in untreated rats (Kaschel et al. 2004). HFS and TPS were repeated twice with the same interstimulus interval that had been used in saturation experiments.

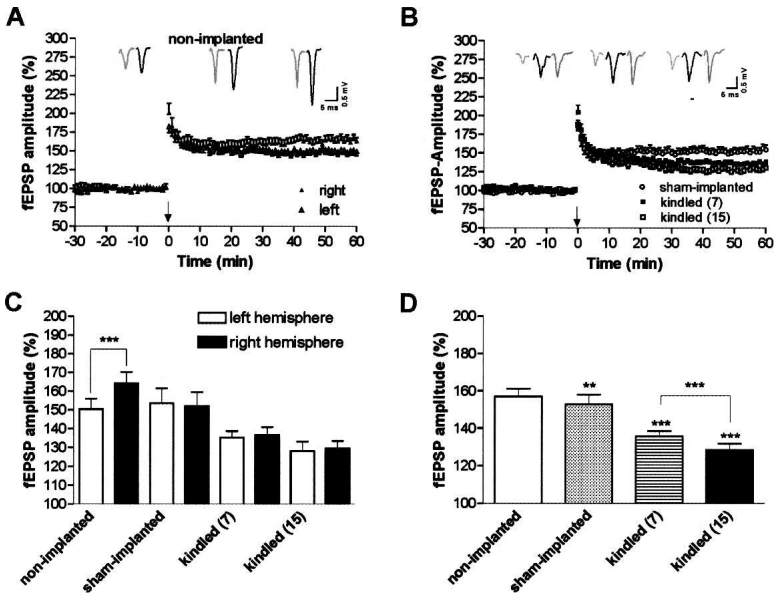

Figure 1. LTP in the LA in respect to hemispheric differences and kindling procedure. (*A,C*) LTP in the left LA and the right LA in slices derived from nonimplanted controls ($n = 32$ slices), sham-implanted controls ($n = 27$ slices), and kindled animals after 7 (K7, $n = 22$ slices) and 15 (K15, $n = 13$ slices) kindling-induced seizures (as indicated in diagrams; electrode implanted into the left BLA). (*A*) Open and closed symbols represent data from the left ($n = 17$ slices, 13 rats) and right hemisphere ($n = 15$ slices, 12 rats), respectively derived from nonimplanted rats. Note the significant difference in LTP between the hemispheres. Data points represent averaged amplitudes (mean ± SEM) of fEPSPs normalized with respect to baseline values. Application of HFS (2 × 100 Hz, interval 30 sec) at time 0. (*C*) Bar histogram of data points in *A* and in sham-implanted (*left*, $n = 12$ slices, 8 rats; *right*, $n = 15$ slices, 12 rats) and kindled animals (K7: *left*, $n = 11$ slices, 7 rats, *right*, $n = 11$ slices, 8 rats; K15: *left*, $n = 8$ slices, 4 rats, *right*, $n = 5$ slices, 3 rats), as averaged 51 to 60 min after HFS and normalized with respect to baseline (mean ± SEM; $^{***}P < 0.0001$). (*B*) Averaged fEPSP amplitudes from sham-implanted controls ($n = 27$ slices) and kindled rats (K7, $n = 22$ slices; K15, $n = 13$ slices). (*D*) Bar histogram of nonimplanted controls ($n = 32$ slices) and data points in *B* as averaged 51 to 60 min after HFS and normalized with respect to baseline (mean ± SEM; $^{**}P < 0.001$; $^{***}P < 0.0001$). Note the seizure-dependent impairment of LA-LTP. Traces show representative examples of evoked fEPSPs in the LA (*A,B*) 5 min before and 20 and 60 min after HFS, respectively.

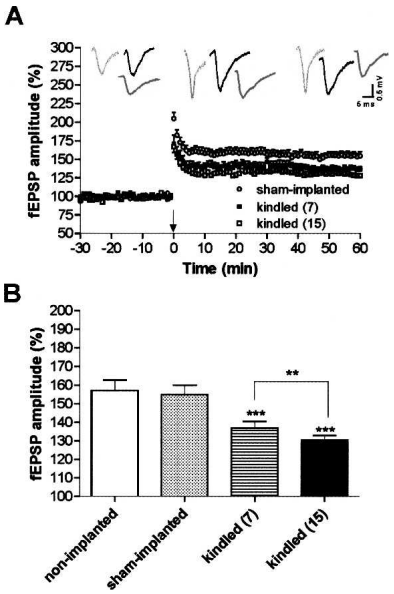

Figure 2. Kindling-induced impairment of CA1-LTP. LTP in the CA1 region of the hippocampus in slices derived from nonimplanted ($n = 21$ slices), sham-implanted ($n = 17$ slices), and kindled rats (K7, $n = 10$ slices; K15, $n = 7$ slices), as indicated near diagrams. (*A*) Data points represent averaged amplitudes (mean ± SEM) of fEPSPs normalized with respect to baseline values. Traces show representative examples of evoked fEPSPs in the CA1 region 5 min before and 20 and 60 min after HFS, respectively. (*B*) Bar histogram of data points in *A* as averaged 51 to 60 min after HFS and normalized with respect to baseline (mean ± SEM; $^{**}P < 0.001$; $^{***}P < 0.0001$). Stimulus protocol as in Figure 1.

Data analysis

Data were collected, and averaged with the custom-made software Signal 2 (Cambridge Electronic Design). Since the slope measure of the evoked population signal in the LA was more sensitive to variability and noise, we decided to compare amplitudes of the evoked signal in the LA and the CA1. We defined the fEPSP as the absolute DC voltage of a vertical line running from the minimal point of the field potential to its intersection with a line running tangential to the points of field potential onset and offset. The group data were then analyzed as follows: (1) the maximum negative fEPSP amplitudes data for each experiment were expressed as percentages of the baseline average (averaged over 4 or 12 min), (2) the time scale in each experiment was converted to time from the onset of HFS, and (3) the time-matched, normalized data were averaged across experiments and are expressed as the means (±SEM). Significance of differences between groups was calculated using the Mann-Whitney test (Software GraphPad Prism 4). $P < 0.05$ was considered significant.

Results

Long-term potentiation

In nonimplanted rats, stable LTP was observed upon HFS in the LA (average increase of fEPSP amplitude 57.1 ± 4.2% above baseline; $n = 32$ slices, 19 rats) with the amplitude in the right LA (64.4 ± 5.8%, $n = 15$ slices, 12 rats) exceeding that in the left LA (50.4 ± 5.6%, $n = 17$ slices, 13 rats; $P < 0.0001$; Fig. 1A). Four to five weeks after implantation of the kindling electrode into the left basolateral nucleus of the amygdala (BLA), stable LTP was preserved in the LA of either hemisphere (Fig. 1B,D), although differences between hemispheres were not observed (right LA: 52.1 ± 7.4%, $n = 15$ slices, 12 rats; left LA: 53.5 ± 7.9%, $n = 12$ slices, 8 rats; Fig. 1C). Therefore, data of both hemispheres were pooled for the following analyses.

Kindling resulted in a significant impairment of LTP in the LA during the postictal period, the magnitude of which was dependent on the number of prior stage V seizures. The data are illustrated in Figure 1B,D. LTP in slices derived from group K7 ($n = 22$ slices, 10 rats) amounted to 35.7 ± 2.8% above baseline, thereby being significantly smaller than that obtained in the sham-implanted controls (52.6 ± 5.3%, $n = 27$ slices, 14 rats; $P < 0.0001$). A further and significant reduction of LTP was observed in the K15 group (28.4 ± 3.5%, $n = 13$ slices, 4 rats; $P < 0.0001$).

In the CA1, HFS-induced LTP was not significantly different in nonoperated rats and sham-implanted controls (57.4 ± 5.5%, $n = 21$ slices, 9 rats vs. 55.1 ± 5.1%, $n = 17$ slices, 7 rats; Fig. 2A,B). Significant hemispheric differences were not observed. As is shown in Figure 2, kindling resulted in a significant reduction in LTP, amounting to 36.9 ± 3.7% and to 30.5 ± 2.4% of the baseline value in slices obtained from the K7 ($n = 9$ slices, 5 rats) and K15 ($n = 7$ slices, 3 rats) groups. The difference between the two groups was significant ($P = 0.0013$), and the LTP obtained in each group was significantly reduced compared to that in sham-implanted controls ($P < 0.0001$).

Figures 1 and 2 also demonstrate that kindling affects different phases of LTP in the CA1 and LA. Whereas only the late phase of LTP was reduced in the LA, the LTP in the CA1 was affected throughout the early and late phases. The data are presented quantitatively in Table 1.

Repeated spaced application of HFS: Saturation of CA1-LTP

The effects of spaced application of the previously used HFS paradigm were analyzed in sham-implanted and kindled animals (K7 group). In the CA1 region of both sham-implanted ($n = 10$ slices,

Table 1. Time course of changes in magnitude of LA-LTP and CA1-LTP in the different animal groups

	LA				CA1			
	non	sham	K7	K15	non	sham	K7	K15
Animals	19	14	10	4	9	7	5	3
N (slices)	32	27	22	13	21	17	9	7
Time (min)								
0–3	78 ± 6	69 ± 7	78 ± 7	66 ± 9	100 ± 12	82 ± 7	57 ± 9*	53 ± 6*
4–7	60 ± 4	51 ± 6	48 ± 5	48 ± 7	70 ± 8	61 ± 6	41 ± 6*	34 ± 4*
8–11	56 ± 5	49 ± 6	46 ± 5	40 ± 5*	68 ± 8	59 ± 7	40 ± 6*	31 ± 3*
17–20	55 ± 5	52 ± 5	41 ± 3*	39 ± 4*	70 ± 7	60 ± 6	40 ± 6*	31 ± 3*
27–30	56 ± 4	51 ± 5	37 ± 3*	30 ± 4*	64 ± 6	59 ± 7	41 ± 6*	34 ± 3*
37–40	55 ± 4	54 ± 5	37 ± 3*	29 ± 4*	60 ± 5	55 ± 6	41 ± 5*	32 ± 2*
47–50	56 ± 4	51 ± 5	36 ± 3*	29 ± 4*	59 ± 6	53 ± 5	37 ± 4*	32 ± 3*
57–60	57 ± 4	53 ± 6	36 ± 3*	29 ± 3*	57 ± 6	55 ± 5	37 ± 4*	29 ± 2*

"non," non-implanted; "sham," sham-implanted; "K7," 48 h after 7 stage V seizures; "K15," 28 h after 15 stage V seizures.

Data are means ± SEM for different time intervals after induction of LTP. An asterisk indicates $P < 0.05$ (comparison of data derived from sham-implanted animals with K7 and K15, respectively). The data show that CA1-LTP means significantly differ for all post-induction time intervals, whereas LTP means in the lateral amygdala did not differ in the early phase of LTP between controls (sham-implanted animals) and kindled rats (K7, K15).

4 rats) and kindled rats (n = 10 slices, 7 rats), saturation of the LTP level was reached after the third HFS application (sham-implanted: HFS1, 58.6 ± 7.4%; HFS2, 75.6 ± 12.6%; HFS3, 92.9 ± 15.7%; HSF4, 86.8 ± 13.8%; kindled (K7): HFS1, 41.5 ± 6.0%; HFS2, 52.4 ± 8.3%; HFS3, 58.3 ± 9.3%; HFS4, 60.6 ± 14.3%; Fig. 3).

LTD and reversal of long-term synaptic plasticity

Since it has been shown that LFS did not result in LTD of BLA neurons in BLA-kindled rats (Wang and Gean 1999), we tested LTD using different paradigms in the LA of BLA-kindled rats. Recently we showed that in young rats a reliable LTD can be induced either by intranuclear LFS or TPS in LA neurons (Kaschel et al. 2004). We found that LTD can also be induced in 4-mo-old rats by TPS ($P < 0.0001$; Fig. 4A,B). The strength of TPS-induced LTD did not differ significantly between nonimplanted (−20.5 ± 6.7%, n = 10 slices, 6 rats) and sham-implanted controls (−19.2 ± 5.6%, n = 8 slices, 3 rats; Fig. 4E). In contrast, in kindled rats (K7) both LFS and TPS caused a long-lasting potentiation of fEPSP amplitudes within the LA (LFS, 43.3 ± 13.3%, n = 6 slices, 3 rats; TPS, 17.2 ± 13.5%, n = 9 slices, 5 rats; $P < 0.0001$; Fig. 4C,D,E).

The TPS-induced LTD could be reversed by subsequent HFS, which resulted in a potentiation of responses above baseline in both nonimplanted (HFS1, 33.2 ± 15.3%; Fig. 4A,B) and sham-implanted rats (HFS1, 12.3 ± 19.6%; Fig. 4C,D). The significant difference of activity after the first HFS presumably reflects an effect of the electrode implantation. Repeating the TPS-HFS application resulted in a significant depression and increase in responses, respectively, in slices obtained from nonimplanted controls (TPS2, 7.1 ± 18.4%; HFS2, 50.1 ± 33.1%; Fig. 4A,B). In sham-implanted rats, the second TPS resulted in no significant change, and the subsequent HFS further increased the responses (TPS2, 10.4 ± 17.7%; HFS2, 44.7 ± 20.6%; Fig. 4C,D). In kindled animals, the application of HFS upon TPS-evoked potentiation resulted in a further significant potentiation of responses (TPS1, 17.2 ± 13.5%; HFS1, 56.4% ± 9.3%; Fig. 4C,D). A second application of TPS-HFS evoked a decrease and increase of the potentiated responses, respectively (TPS2, 39.9 ± 12.2%; HFS2, 53.2 ± 11.2%; Fig. 4C,D).

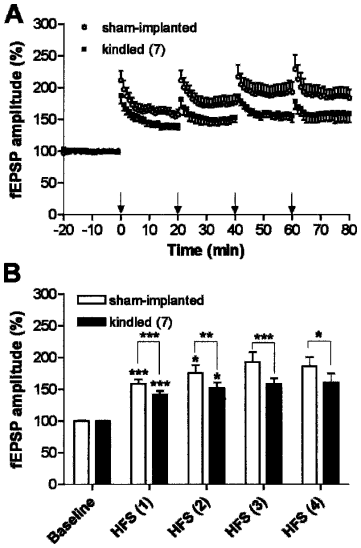

Figure 3. Saturation level of CA1-LTP in kindled rats (K7, n = 10 slices, 4 rats) compared to sham-implanted animals (n = 10 slices, 7 rats) in response to repeated spaced high-frequency stimulation (4 × 2 × 100 Hz, interval 30 sec with the spaced interval of 20 min). (*A*) Data points represent averaged amplitudes (mean ± SEM) of fEPSPs normalized with respect to baseline values. (*B*) Bar histogram of data points in *A* as averaged 56 to 60 min after HFS and normalized with respect to baseline (mean ± SEM; *$P < 0.05$; ***$P < 0.0001$). Note the same saturation level in sham-implanted and kindled rats.

Discussion

The results of the present study indicate that kindling of the left BLA resulted in a significant impairment in the overall magnitude of LTP in both the lateral nucleus of the amygdala (LA) and the hippocampus (CA1), the magnitude of which was dependent on the number of prior stage V seizures. Hemispheric differences observed in LA-LTP in nonimplanted controls disappeared after electrode implantation as well as after kindling. Further, LTP was evoked upon both LFS and TPS in the LA in kindled animals, whereas TPS depressed activity in nonkindled controls.

Effect of kindling procedure on LTP magnitude

It was shown previously that the induction of LTP in the CA1 region is strongly depressed if the inducing stimulus is delivered during the period of postictal depression (a 5–10-min period of field response depression) following an evoked electrographic seizure (Barr et al. 1997). Further, electroconvulsive treatment markedly inhibited HFS-induced LTP in the hippocampus (Anwyl et al. 1987). Moreover, in TLE patients, *N*-methyl-D-aspartate (NMDA)-dependent LTP was strongly suppressed in the dentate gyrus compared to nonepileptic tissue (Beck et al. 2000). We showed previously that HFS-induced LA-LTP is NMDA-dependent in horizontal slices of adult untreated rats (Albrecht et al. 2002), whereas nifedipine, an L-type calcium antagonist, did not influence the LTP in the LA. These data correspond with data from coronal slices where thalamo-amygdala HFS-induced LTP was only dependent on NMDA receptors (Bauer et al. 2002). In contrast, it is known that L-type calcium channels may be involved in the mediation of CA1-LTP in adult rats, in addition to NMDA receptors (Cavus and Teyler 1996; Matias et al. 2003). These or other differences in signal cascades may be responsible for the different time courses of LTP curves in the amygdala and the hippocampus in kindled rats compared to controls.

The results of the present study are in line with and add to the previously reported notions that LTP in both the CA1 and LA is impaired after kindling, and that the degree of impairment is related to the severity of kindling-induced seizures. That these changes in LTP magnitude relate to epileptic seizure activity and did not represent an acute effect is corroborated by the observation of a similar impairment of LTP in pilocarpine-treated animals (Schubert 2005).

Although a significant reduction of cell density and the appearance of degenerated fibers was evident in the LA after 15 stage V seizures in BLA-kindled rats (von Bohlen und Halbach et al. 2004), our pharmacological investigations in intracellular recordings showed that kindling also caused changes in GABAergic and glutamatergic transmission (Albrecht and Schubert 2004).

Kindling-related masking effect versus changes in LTP properties

The results obtained with repeated spaced HFS indicate that a change in LTP threshold and/or saturation rather than a masking effect underlies the impairment of LTP after kindling. It is commonly observed that repeated delivery of HFS leads to a gradually increasing degree of induction of LTP up to saturation, and that an excessively large number of stimulus trains is deleterious to LTP (Abraham and Huggett 1997). Our results confirm the deleterious effect of excessive stimulation.

Upon LFS, robust LTD is readily induced in hippocampal

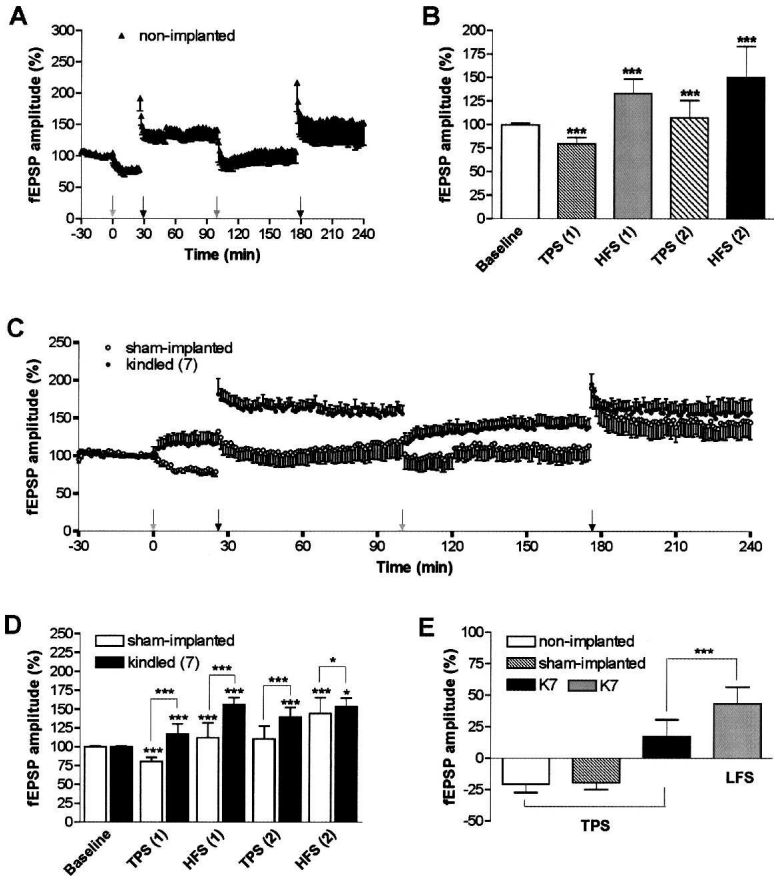

Figure 4. Effects of repeated TPS, LFS, and HFS in the LA: differences among nonimplanted, sham-implanted, and kindled rats. (*A*) LTD and its reversal in the LA in slices derived from nonimplanted rats ($n = 10$ slices, 6 rats). Data points represent averaged amplitudes (mean ± SEM) of fEPSPs normalized with respect to baseline values. Application of TPS (8 Hz, 150 sec; gray arrows) and of HFS (2 × 100 Hz, interval 30 sec, black arrows) twice. (*B*) Bar histogram of data points in *A* as averaged 51 to 60 min after TPS and HFS and normalized with respect to baseline. (*C*) The same TPS paradigm as used in *A* caused a potentiation of fEPSP amplitudes in kindled rats (K7, $n = 9$ slices, 5 rats), whereas it depressed the activity in sham-implanted rats ($n = 8$ slices, 3 rats). HFS facilitated TPS-induced LTP in kindled rats, whereas it reversed LTD in sham-implanted rats. The second TPS induced a weak LTD in kindled rats, whereas it did not significantly change the activity in sham-implanted animals. The second HFS caused in both animal groups an enhancement of activity. (*D*) Bar histogram of data points in C as averaged 51 to 60 min after TPS and HFS and normalized with respect to baseline, $^*P < 0.05$; $^{***}P < 0.0001$. (*E*) Effects of TPS and LFS on fEPSP amplitudes normalized with respect to baseline in the LA of slices derived from nonimplanted, sham-implanted, and kindled animals. Note that LFS caused a stronger enhancement of activity in kindled rats compared to TPS.

slices from very young animals, but not or to a lesser degree in adult animals (Battistin and Cherubini 1994; Kemp and Bashir 1999). In the LA, LTD can be induced by both TPS and LTS in juvenile (Heinbockel and Pape 2000; Kaschel et al. 2004) and adult animals (present study). We have shown that LTD induced in horizontal slices is dependent on NMDARs as well as on group II metabotropic glutamate receptors (Kaschel et al. 2004). Kindling dramatically changes the responses to TPS and LFS. Whereas in nonimplanted and sham-implanted rats the TPS-induced LTD could be reversed by HFS, the strength of TPS-induced LA-LTP was further increased by the following HFS in kindled animals. That the balance between threshold and saturation may be affected by kindling-evoked seizure activity is indicated by the significant alteration in both magnitude and polarity of plasticity once it has been induced.

Although the mechanisms that link seizure activity to changes in long-term plasticity cannot be deduced from the existing data, it is interesting to note that the intracellular calcium dynamics are involved in both processes (McEachern and Shaw 1999). Epileptic seizure activity is associated with significant changes in calcium influx, intracellular calcium concentration, and calcium-dependent secondary mechanisms (Heinemann et al. 1990; Faas et al. 1996). Abraham and Huggett (1997) showed that in the CA1 region of the hippocampus, overstimulation transiently inhibits subsequent LTP induction through activation of voltage-gated calcium channels (VGCCs) and of NMDA receptors. Further, the intracellular calcium concentration seems to be a crucial determinant of the polarity and magnitude of long-term synaptic plasticity, in that low levels of calcium via activation of calcium-dependent phosphatases facilitate LTD, whereas a shift towards higher calcium levels triggers LTP via protein kinase pathways (Kirkwood and Bear 1995). Kindling-evoked seizure activity may thus prime synapses via calcium-dependent mechanisms, thereby affecting the threshold, magnitude, and saturation of long-term plasticity at these synapses. This sort of metaplasticity may then contribute to the alteration in memory performance and emotional behavior observed in TLE patients (Van Elst et al. 2000; Hecimovic et al. 2003; Helmstaedter et al. 2004; Johnson et al. 2004). These results may also help to explain on the level of synaptic plasticity previous observations regarding nonepileptic behavioral alterations after kindling (Hannesson and Corcoran 2000) and, more specifically, the finding that kindling-induced fear increases with the number of seizure-provoking stimulations (Kalynchuk et al. 2001).

Hemispheric differences

It is now widely accepted that the amygdala is an essential element for the acquisition of conditioned fear as well as for the expression of innate and learned fear responses, and that emotional perception is differentially regulated by the hemispheres (LeDoux 2000; Dolan 2002). For instance, it has been shown that short-term kindling of the right BLA has anxiogenic effects, whereas short-term kindling of the left BLA has anxiolytic effects (Adamec and Morgan 1994). It is also known that an asymmetrical seizure-induced breakdown of the blood-brain barrier can be obtained in rats (Kutlu et al. 2002). Neurotransmitter systems are also lateralized (Reynolds et al. 1990; Andersen and Teicher 1999; Thiel and Schwarting 2001). It was recently shown that the synaptic distribution of NMDA receptor epsilon2 (NR2B) subunits in the adult mouse hippocampus is asymmetrical between the left and right and between the apical and basal dendrites of single neurons. These asymmetrical allocations of epsilon2 subunits differentiate the properties of NMDA receptors and synaptic plasticity between the left and right hippocampus (Kawakami et al. 2003). In humans, the hypothesis of right hemisphere dominance for emotional functions is supported by a very large body of clinical and experimental evidence (Baas et al. 2004; Baker and Kim 2004). The observation made in the present study that the magnitude of LTP in the right LA exceeds that in the left LA provides another example of hemispheric lateralization of transmitter systems, and may provide a further link between long-term synaptic plasticity in the amyg-

dala associated with the formation of Pavlovian fear memory (Rogan et al. 1997) and the lateralization of these functions.

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